Polish Journal of Environmental Studies Vol. 11, No. 4 (2002), 325-330

Potentially Pathogenic Bacteria from the Family *Enterobacteriaceae, Pseudomonas* sp. and *Aeromonas* sp. in Waters Designated for Drinking and Household Purposes

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Received: 25 April, 2001
Accepted: 7 January, 2002

Abstract

This work describes bacteriological studies on the quantitative and qualitative composition of bacteria from the family *Enterobacteriaceae* as well as numbers of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in underground waters of the Omulewski Reservoir, a source of water for consumption. In 1994-1995 studies were carried out on 11 deep wells and 3 piezometric holes. Bacteria from the family *Enterobacteriaceae* were found in amounts from a few to many colonies (cfu/100 ml), depending on the sampling station and the method of land use (groups of wells). In the entire study period and usually in all wells the following dominated: *Citrobacter freundii, Ervinia herbicola, Escherichia coli, Proteus vulgaris,* and *Serratia marcescens. Pseudomonas aeruginosa* and *Aeromonas hydrophila* were present in small amounts in the majority of water samples (from a few to several colonies) and only when large quantities of water were collected (100 ml).

Keywords: underground waters, potentially pathogenic bacteria, *Enterobacteriaceae, Pseudomonas aeruginosa, Aeromonas hydrophila*

Introduction

Increasing amounts of discharged sewage, progressing urbanisation, the chemicalization of agriculture and industry, as well as anthropogenic activities all affect the quality of underground waters. The final effect of water degradation are the limits as to the use of drinking water reservoirs. Frequently this state is coupled with microbiological contamination, resulting in the penetration of potentially pathogenic bacteria or microorganisms detrimental to underground waters through the soil [26]. Hence, these bacteria may become the source of various diseases, the intensity of which would largely depend on microorganism pathogeneity and disease potential. Numbers of bacteria are also important, as well as their survival and possibilities to adapt and migrate deep into water-bearing underground reservoirs [23]. Some of the bacteria, such as Pseudomonas or *Aeromonas,* may be a threat to human health due to their ability to multiply in drinking waters [12]. Others, especially those which constitute natural microflora of human and animal food tracts, can induce acute or chronic gastric diseases [22].

Bacteriological contamination is most dangerous in the case of shallow reservoirs of underground water originating from the Quarternary. These reservoirs represent over 50% of all Polish water resources [2]. They are

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characterised by shallow location and small thickness of the water-bearing layers, as well as direct impact of atmospheric precipitation. Due to this fact they are most threatened by various pollutants [17]. Omulewski Reservoir is a good example of such water bodies, with most unfavourable conditions for natural protection [27]. Until 1992 the reservoir was under strong anthropopression resulting from intensive agricultural management in the area (unbedded rearing of domestic animals). Earlier chemical [13, 15] and sanitary and bacteriological studies [20] showed that waters of this reservoir should also be monitored as regards potentially pathogenic bacteria. Consequently, the aim of the research was to monitor the supplies of drinking water from the Omulewski Reservoir, especially in terms of quantitative and qualitative frequency of bacteria from the family *Enterobacterieae* and the species *Pseudomonas aeruginosa* and *Aeromonas hydrophila* as potential waterborne pathogens and health risk factors for inhabitants of Warmia and Mazury.

Materials and Methods

Study Area

Studies were performed in Omulewski Reservoirs (Fig. 1.). Details of its location, hydrogeological structure, and criteria of selecting and locating particular sampling stations were presented in earlier papers [14, 15, 20]. Characteristics of the 11 deep wells and 3 piezometric holes are presented in Table 1.

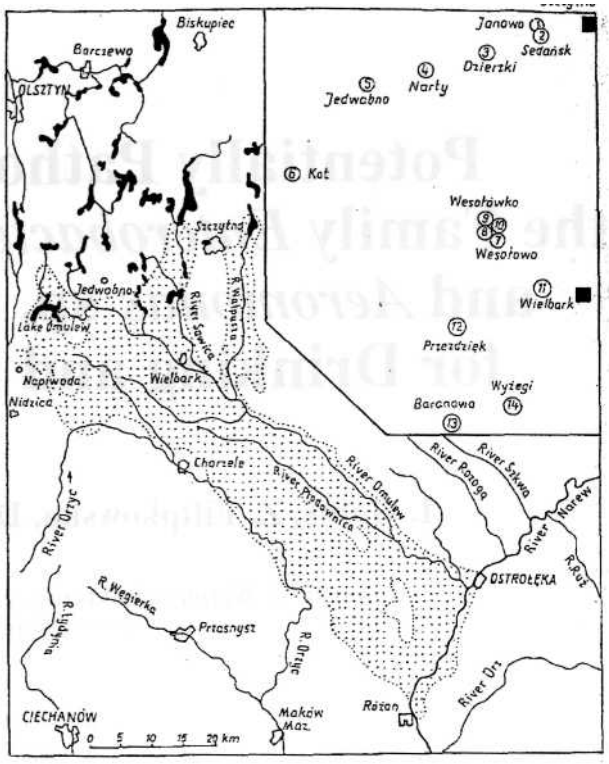

Fig. 1. Location sampling stations of underground waters from Omulewski Reservoir according to Szczepkowski [23].

Table 1. Characteristics of wells and piezometric holes in Omulewski Reservoir according to Niewolak [20] after Kochańska [13].

Group of wells	Location	User	Well depth	Type of water
I	Sedańsk	Fox farm	38.0	CO_3/Ca/Mg [1]
	Narty	Forest house	40.0	CO_3/Ca/Mg
II	Jedwabno	Dairy	49.0	–
	Kot	Polyethylene Recycling Factory	20.0	CO_3/Ca [2]
III	Janowo	Fox farm (2 000)*	42.0	CO_3/Ca/Mg
	Dzierzki	Pig farm (2 500)	60.0	CO_3/Ca/Mg
	Wesołowo	Cow farm (700)	38.0	CO_3/Ca
	Wielbark	Pig farm (10 000)	60.0	CO_3/Ca/Mg
	Przeździęk	Cow farm (500)	34.0	CO_3/Ca
	Baranowo	Cow farm (500)	64.0	CO_3/Cl/Ca [3]
	Wyżegi	Cow farm (1 000)	25.0	CO_3/Ca/SO_4 [4]
IV	Wesołowo – piezometric hole no. 1	Cow farm	6.8	
	Wesołowo – piezometric hole no. 2	Cow farm	23.5	
	Wesołowo – piezometric hole no. 3	Cow farm	21.5	

* - in parenthesis number of animals bred until 1992;
[1] - water of carbonate-calcium-magnesium type;
[2] - water of carbonate-calcium type;
[3] - water of carbonate-chloride-calcium type;
[4] - water of carbonate-calcium-sulphate type.

Table 2. Bacteria from the family Enterobacteriaceae in underground waters of Omulewski Reservoir in 1994-1995.

Species	Sampling stations													
	Control wells located in afforested areas		Wells located over lands used by individual farmers		Wells and piezometric holes located over the lands intensively used for animal breeding									
	Sedańsk	Narty	Jedwabno	Kot	Janowo	Dzierzki	Wesołowo	Wielbark	Przeździęk	Baranowo	Wyżegi	Piezometric hole no. 1	Piezometric hole no. 2	Piezometric hole no. 3
	Number of colonies (cfu/100 ml)													
Cedacea davisae	2[a] 0 – 3[b]	1 0 – 2	–	–	–	1 0 – 3	1 1 – 4	1 0 – 3	1 0 – 2	1 0 – 3	1 0 – 3	3 1 – 5	2 0 – 8	1 0 – 2
Cedacea lapagei	–[c]	–	1 1 – 2	2 0 – 5	2 0 – 4	3 1 – 5	3 1 – 5	–	2 0 – 6	4 1 – 5	1 0 – 4	3 0 – 6	1 0 – 3	–
Citrobacter freundii	–	1 0 – 2	2 1 – 4	1 1 – 3	7 1 – 12	14 3 – 54	10 5 – 68	2 0 – 7	10 3 – 112	12 3 – 24	16 4 – 110	16 4 – 146	13 5 – 103	10 2 – 73
Citrobacter diversus	3 4 – 7	2 0 – 6	–	1 0 – 3	2 0 – 4	3 2 – 10	5 0 – 26	2 0 – 9	5 3 – 17	3 0 – 5	2 0 – 8	5 1 – 17	4 1 – 10	3 1 – 4
Edwardsiella tarda	1 0 – 4	1 0 – 2	–	–	1 0 – 3	2 0 – 4	–	–	1 0 – 2	1 0 – 3	1 0 – 4	2 0 – 8	2 0 – 6	2 0 – 1
Enterobacter agglomerans	–	–	21 8 – 43	8 1 – 16	2 0 – 5	–	3 0 – 18	6 1 – 25	4 2 – 10	2 1 – 3	2 0 – 8	8 1 – 21	5 3 – 9	3 1 – 5
Enterobacter aerogenes	–	2 0 – 4	–	3 1 – 6	1 0 – 3	–	1 0 – 2	7 2 – 34	–	1 0 – 2	1 0 – 3	12 3 – 47	9 1 – 29	9 3 – 17
Enterobacter cloaceae	–	–	1 0 – 2	–	–	–	–	–	–	–	–	10 1 – 29	7 0 – 38	6 1 – 22
Enterobacter intermedium	–	–	10 3 – 16	1 0 – 2	2 0 – 5	1 0 – 3	3 0 – 9	10 2 – 54	6 3 – 10	3 1 – 7	2 0 – 9	–	–	–
Ervinia herbicola	2 1 – 5	2 0 – 7	2 0 – 5	5 0 – 10	2 0 – 6	1 0 – 2	6 1 – 21	18 5 – 98	7 4 – 15	1 0 – 2	4 1 – 14	4 2 – 17	3 0 – 11	4 1 – 8
Escherichia coli	1 1 – 4	3 1 – 5	2 0 – 6	3 1 – 7	5 1 – 9	5 2 – 7	17 3 – 81	3 0 – 8	9 6 – 21	6 1 – 11	3 2 – 8	20 7 – 198	16 4 – 94	12 4 – 52
Klebsiella ozaenae	1 0 – 2	–	1 0 – 2	–	8 1 – 14	6 1 – 12	9 2 – 72	13 3 – 74	3 0 – 8	2 0 – 2	1 1 – 2	5 0 – 10	1 0 – 2	1 0 – 3
Kluyvera ascorbata	2 1 – 3	–	1 0 – 3	–	2 1 – 5	1 0 – 3	2 1 – 4	3 0 – 6	4 1 – 9	1 0 – 3	3 0 – 10	1 0 – 3	1 0 – 3	1 0 – 3
Proteus vulgaris	2 1 – 5	–	2 1 – 4	2 0 – 5	1 2 – 17	8 4 – 23	12 3 – 85	13 1 – 75	14 2 – 85	16 3 – 98	19 0 – 94	22 5 – 294	17 4 – 105	14 1 – 83
Serratia marcescens	–	2 1 – 4	11 3 – 20	7 1 – 15	19 2 – 69	7 2 – 18	16 1 – 105	15 2 – 132	18 4 – 93	12 5 – 85	16 0 – 78	4 1 – 10	3 1 – 5	3 2 – 6
Serratia rubidea	–	1 0 – 3	–	3 0 – 8	5 1 – 11	8 4 – 21	3 0 – 10	15 4 – 112	2 1 – 5	6 2 – 130	5 0 – 14	8 2 – 27	6 0 – 15	1 0 – 3
Yersinia enterocolitica	–	–	–	2 0 – 4	1 0 – 3	3 1 – 5	2 1 – 5	2 0 – 6	–	–	–	1 0 – 2	–	–
Totally	**12** **0 – 40**	**15** **0 – 72**	**52** **4 – 148**	**38** **7 – 106**	**66** **0 – 157**	**63** **0 – 128**	**93** **1 – 232**	**110** **3 – 278**	**86** **0 – 142**	**71** **1 – 136**	**77** **0 – 156**	**124** **10 – 387**	**90** **11 – 174**	**70** **7 – 110**

[a] – mean, [b] – range, [c] – not found

Sampling

The underground waters of the Omulewski reservoir were studied in 2-year-long cycles. Underground water samples were collected from the wells from March 1994 until November 1995, at 3-month intervals. Water samples from particular stations were collected according to the methods described by Niewolak [20].

Bacteriological Examination

Bacteriological assays included determination of the qualitative and quantitative composition of bacteria of the family *Enterobacteriaceae* and the count of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in the water of wells and piezometric holes of the Omulewski Reservoir. The analyses were performed as follows:

- *Enterobacteriaceae* - 100 ml water samples were fil tered through SYNPOR-rype membrane filters (no 2, 50 µm in diameter). After filtering, the filters were placed on plates with Endo selective medium [6] and incubated at 37°C for 48 hours, after which all colonies were counted. For identification, all colonies which differed morphologically were Gram negative and subject to a test on the presence of cytochromic oxidase. All Gram nega tive and oxidase negative strains were screened for identification tests - ENTEROPLASTS® (produced by PLASTOMED). Based on the results, genera and species of the bacteria of the family *Enterobacteriaceae* were de termined. A percentage of all species (isolates) in the total count of the bacteria grown on Endo medium was computed;
- *Pseudomonas aeruginosa* - 100 ml water samples were densified on membrane filters as described above and placed on King A selective medium [6] and incu bated at 24°C for 24 hours. After that, milk-white colo nies growing on the medium surface and shining under a Wood lamp were counted [1]. Further identification of those colonies was carried out according to the data from the references: Dutka and Kwan [7] and Krueger and Sheikh [16];
- *Aeromonas hydrophila* - 100 ml water samples were densified on membrane filters as described above. The filters were then placed on mA selective medium [24] and incubated at 37°C for 20 hours. After that, yel low-coloured colonies were counted and further identifi ed according to methods cited in the literature [11].

In 1994-1995 these studies were made on 106 samples of underground water from Omulewski Reservoir. Only once, in 1995, was no sample collected from the well in Sedansk due to hydrophore repairs.

Results

Quantitative and qualitative composition of bacteria from the family *Enterobacteriaceae* for the wells under study is presented in Table 2. Their number in 100 ml of water ranged from 0 colonies in the wells located in Sedansk, Narty, Janowo, Dierzki and Przezdziek to a few hundred colonies in underground waters of other stations. When the mean is taken into account, the lowest qualitative and quantitative variations were found in the control wells located in afforested areas (Sedanska, Narty), slightly higher in two wells in the areas used by individual farmers (Jedwabno, Kot), and the highest in waters of other wells and piezometric holes located in area used for animal production. The following species usually dominated in all samples: *Citrobacter freundii, Ervinia herbicola, Escherichia coli, Proteus vulgaris* and *Serratia marcescens.* Other species from the family *Enterobacteriaceae* were rare or found in very small numbers. Among the strains of bacteria isolated and identified in 100 ml water, no cells of *Salmonella* and *Shigella* were detected.

Per cent contribution of particular species of the family *Enterobacteriaceae* throughout the whole research period is presented in Table 3. Most of those species constituted between 1.3 and 7% of all the strains. The following species were dominant in total microbiological

Table 3. Percent of viable species of bacteria from the family *Enterobacteriaceae* in underground waters of Omulewski Reservoir in 1994-1995.

Species	Percentage
Cedacea davisae	2.6[a] 0 – 17.0[b]
Cedacea lapagei	2.4 0 – 7.0
Citrobacter freundii	11.0 0 – 22.0
Citrobacter diversus	6.0 0 – 26.0
Edwardsiella tarda	2.2 0 – 8.0
Enterobacter agglomerans	7.0 0 – 38.0
Enterobacter aerogenes	4.7 0 – 13.0
Enterobacter cloaceae	1.9 0 – 9.0
Enterobacter intermedium	3.7 0 – 18.0
Ervinia herbicola	6.6 1.0 – 16.0
Escherichia coli	10.7 3.0 – 16.0
Klebsiella ozaenae	4.7 0 – 12.0
Kluyvera ascorbata	2.4 0 – 8.0
Proteus vulgaris	13.2 0 – 25.0
Serratia marcescens	13.7 0 – 29.0
Serratia rubidea	5.9 0 – 14.0
Yersinia enterococolitica	1.3 0 – 5.0
Totally	100.00

[a] – mean [b] – range

contamination: *Serratia marcescens* (13.7%), *Proteus vulgaris* (13.2%), *Citrobacter freundii* (11%) *and Escherichia coli* (10.7% of all the identified strains). *Yersinia enterococolitica* and *Enetrobacter cloaceae,* on the other hand, accounted for the lowest percentages (1.3 and 1.9% of all the strains, respectively).

Pseudomonas aeruginosa and *Aeromonas hydrophila* were rare (a few colonies in 100 ml of water), but in the majority of deep wells. The highest numbers (several colonies in 100 ml of water) were found in water samples from piezometric bore holes (Tab. 4).

Table 4. Presence of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in waters of Omulewski Reservoir in 1994-1995.

Sampling station	*Pseudomonas aeruginosa*	*Aeromonas hydrophila*
	Number of colonies (cfu/100 ml)	
I. Control wells located in afforested land:		
Sedańsk	–[a]	–
Narty	1[b] 0 – 2[c]	2 0 – 3
II. Wells located in land used by individual farmers:		
Jedwabno	3 1 – 4	1 0 – 2
Kot	3 0 – 6	–
III. Wells and piezometric holes in lands used for intensive animal breeding:		
Janowo	3 0 – 7	2 0 – 5
Dzierzki	2 0 – 4	–
Wesołowo	3 1 – 6	4 1 – 8
Wielbark	6 2 – 13	1 0 – 2
Przeździęk	1 0 – 2	3 1 – 7
Baranowo	2 1 – 5	1 0 – 2
Wyżegi	2 1 – 4	4 1 – 8
Piezometric hole no.1	15 7 – 32	12 9 – 25
Piezometric hole no. 2	12 4 – 28	11 7 – 21
Piezometric hole no. 3	17 10 – 29	10 4 – 36

[a] – not found, [b] – mean, [c] – range.

Discussion

Qualitative and quantitative composition within the family *Enterobacteriaceae* in samples of underground waters of Omulewski Reservoir were probably due to unfavourable hydrological conditions, namely to shallow location of water-bearing layers and lack of isolation from the surface [27]. Differences in the quantitative frequency of *Enterobacteriaceae* (depending upon previous use of the land surface) confirm the long-term effect of intensive farming and animal breeding on the quality of those waters [13,15], associated with the sandy structure of soils, mean values of the hydraulic gradient (I_{mean}) and filtration factor (K_{mean}) [10].

Agricultural organic pollutants deposited in the soil (in these, potentially pathogenic bacteria and pathogens as such) may migrate with precipitation waters into water-bearing layers [4, 5, 19], thereby deteriorating the bacteriological state of waters and increasing the risk of spreading various diseases [3]. In the sanitary and bacteriological analyses of the underground waters of the Omulewski Reservoir including TC and FS determination, higher TC and FS values were obtained, ranging from 0 to 18 and from 4 to 75 cells in 100 ml, respectively [21]. This corresponded to higher numbers of bacteria of the family *Enterobacteriaceae,* which was specially evident in the waters of piezometric holes. This could be explained by the sandy soil structure and the effect of intensive breeding technologies implemented on this area until 1992 [10, 13, 15]. Such bacteriological status of the reservoir poses a threat of transmission of waterborne diseases.

Our results show that some *Enterobacteriaceae* species such as *Serratia, Proteus, Citrobacter,* and *Escherichia,* are clearly dominant. Their prevalence was also confirmed by qualitative analyses of the heterotrophic microflora of the Omulewski reservoir, which revealed high counts of bacteria of *Citrobacter, Proteus* and *Serratia* species [9]. The qualitative composition of bacteria of the family *Enterobacteriaceae* in the underground waters of the Omulewski Reservoir is similar to the literature data on other reservoirs of underground waters.

Qualitative composition of bacteria from the family *Enterobacteriaceae* in underground waters of Omulewski Reservoir was close to the literature data. Shirey, Bissonnette [25] studied underground waters and found that they were dominated by: *Eschericha coli, Enterobacter agglomerans* and *Klebsiella pneumoniae.* Similar results were obtained for 220 wells studies by Franzblau et al. [8].

Pseudomonas aeruginosa and *Aeromonas hydrophila* were sporadic and low counts in the wells under study. Similar to the case of the family *Enterobacteriaceae* were most abundant in waters of piezometric holes, as those are the shallowest and the most susceptible to pollutants [13, 20]. According to literature data [11, 24, 28], these were the species commonly present in aquatic environments. However, their higher numbers may represent a real threat to human health [18].

Summary

The results of studies on the numbers of potentially pathogenic bacteria in Omulewski Reservoir suggest that

this reservoir of drinking water was very susceptible to pollutants, especially those of local character. Higher counts of bacteria from the family *Enterobacteriaceae* were found in wells and piezometric holes located in places where there used to be intensive animal rearing. This could have been the result of bacteriological contamination. Considerably high numbers of bacteria of the family *Enterobacteriaceae,* determined mainly in waters of the wells and piezometric holes situated on former cattle and swine farms, are indicative of high rates of bacteriological contamination and could be a cause of the spread of bacterial diseases. Accordingly, there is a risk that such diseases will spread among the local population. It therefore seems advisable to continue research on qualitative composition of potentially pathogenic microflora.

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